EVALUATION OF PHYTOCHEMICAL, ANTIOXIDANT AND ANTI-CANCEROUS ACTIVITY OF *LANTANA CAMARA* L. AND *URGINEA INDICA* (ROXB.) KUNTH

SYNOPSIS OF THE THESIS

Submitted by

Banita Dhatwalia

(Registration No. AU20ZOPhD0046)

Under supervision of

Dr. (Mrs.) Neetu Sharma

In Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

IN

ZOOLOGY

SCHOOL OF BASIC SCIENCES

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ABHILASHI UNIVERSITY

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CERTIFICATE

This is to certify that the synopsis of the thesis titled **"Evaluation of phytochemical, antioxidant and anti-cancerous activity of *Lantana camara* L and *Urginea indica* (roxb.) Kunth"** is an original piece of work done by Ms. Banita Dhatwalia (Registration No. AU20ZOPhD0046), under my supervision for the degree of Doctor of Philosophy (Zoology) in Abhilashi University Chail-Chowk Mandi, Himachal Pradesh. It is also certifed that this work has not been submitted to any other University for a Ph.D. degree.

Place: Chail chowk

Date: 28/06/22

(Signature of the Supervisor)

Dr Neetu Sharma

(Name of the Supervisor)

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Place: **(Signature of the Supervisor)**

Date: **(Name of the Supervisor)**

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A. Introduction

Globally cancer is the most life taking disease that is increasing due to our daily life style (Mathur *et. al.,* 2015). It is a disease in which some of the cells of human body divide uncontrollably at any part of body (National Cancer Institute, 2021). Worldwide cancer is the most death dealing disease that results in a count of approximately 10 million of deaths in 2020 or an average of one in six deaths. Lung cancer, liver cancer and breast cancer are the most common type of cancer detected regularly. In year 2020, 2.26 million cases of breast cancer, 2.21 million cases of lung cancer were reported. Whereas 1.80 million deaths due to lung cancer, 830,000 deaths due to liver cancer and 685,000 deaths due to breast cancer were reported (WHO Report, 2021). Our daily lifestyle has an essential role in a global increase of breast cancer cases. An unfavorable lifestyle leads to an estrogen-dependent mechanism which causes proliferation of carcinogenic cells. Therefore, one may hypothesize those daily life activities like tobacco smoking increase the risk of positive breast cancer (Trygve *et. al.,* 2020). Lung cancer happens when cells mutate rapidly. Various factors can cause this mutation like smoking, radon exposure, particle pollution, hazardous chemicals (uranium, cadmium, chromium, nickel etc.) (American Lung association, 2021). On other hand, daily life pattern also appear to play an essential role to increase the risk for hepatocellular carcinoma. Polyunsaturated fat, red meat, saturated fat and cholesterol consumption related to increase the risk of liver cancer. An excessive amount of alcohol and tobacco are directly associated with carcinogenic effect (Shira *et. al.,* 2021) and indirectly results in various types of conditions like cirrhosis, necrosis, liver carcinoma etc. (Wang *et. al.,* 2017; Jannu, 2012).

However, there are several of medicinal preparations in our traditional systems like Ayurveda and Homeopathy that are suggested for the treatment of a number of human health issues (Yogi and Mishra, 2017, Kumar *et al*., 2015). For the treatment of cancerous diseases, plants having medicinal values are remarkable source for the finding of anti-cancerous drugs. Numerous drugs derived from the traditional plants are used for medical purpose (Jain *et al*., 2015; Gupta *et al.,* 2014). Although, a couple of plants used in traditional medicine are phytochemically evaluated for their safety and ability, and many are yet to be examined (Kumar, 2015). Therefore, the recognition of therapeutic ability of medicinal plants is necessary.

Lantana camara (**Linn**.) is a traditional medicinal plant belongs to family Verbenaceae (Kalita *et al*., 2011). It is considered to be a weed plant mainly found in

forests but is also a garden plant. *Lantana camara* is commonly known as “Tick berry”, “Spanish flag” and “Wild-sage”. The leaves of the plant are usually used as a herbal medicine by many Asian and Middle Eastern countries. Its leaves are known to treat skin allergy, wounds and ulcers also. Antioxidants as well as anti-cancerous activities are found in the leaves extraction of the plant.

Lantana camara

Urginea indica is an essential herbal plant belongs to family Liliaceae. It founds abundantly in the forests, rocky, hilly and moist areas of India. *Urginea indica* is also known as “Sea Onion”, “Indian Squill” and “True Squill”. In India it is commonly called as “Bon Piaj” or “Jungli Piyaz”. As it is an indigenous plant, it plays a prime part in the discovery of several drugs. Generally the bulbs of the plant are popular for the treatment of cough, cold, fever, measles, headaches and pneumonia. A number of experimental studies have examined antiviral, anti-bacterial, antioxidant, anti-cancerous, anti-inflammatory potential of *Urginea indica*.

Urginea indica

B. Review of Literature

Lantana camara* (*Linn.) is an exotic weed that is also known as "Tickberry". It belongs to family Verbenaceae and found in forest ecosystem, mainly seen in hilly areas (Gulshan and Hem, 2021). In Himachal Pradesh it is commonly called as "Phulnu" or "Panchphulli" and found in Mandi, Hamirpur, Nahan and Bilaspur districts (Himachal Pradesh Forest Department). Experimental analysis examined the presence of many flavonoids, glycosides and alkaloids in *Lantana camara* such asaliphatic and oxygenated hydrocarbons and mono and sesquiterpenes (sabinene, cis-Ocimene, Eucalyptol, Geranyl isovalerate, Menthol-1'-(butyn-3-one-1-yl), 7-epi-trans-sesquisabinene Hydrate, trans-Caryophyllene, tau.-Cadinol and Limonen-6-ol-pivalate (Joslen and Ahmed, 2018). The plant extract showed anticancer potential against different types of cancer cells like human breast cancer cell line, MCF-7. It also examined that the cell death induced by treatment with the *Lantana. camara* extract was regulated by the Bcl-2 family. Bcl-2 was decreased and Bid and Bax was increased by using *Lantana. camara* extract. The plant extracts balanced cleavage of caspase-8, and caspase-9, as well as poly (ADP-ribose) polymerase (PARP) (Han *et. al.,* 2014). The study supports the possible application of the *Lantana camara* extract as an anti-cancer drug.

Urginea indica is a herbal plant (Family Liliaceae) that is locally known as "Jungli Piyaz". It is mainly found in western Himalaya, Bihar and foot hill regions of Himachal Pradesh: Bilaspur, Mandi and Hamirpur. Traditionally, it has been used to cure cough, respiratory disorders, ear ache and cold (Gulshan and Sampy, 2019). A number of experimental studies have examined antiviral, anti-bacterial, antioxidant, anti-cancerous, anti-inflammatory potential of *Urginea indica (*Kameshwari *et. al.,* 2012). Some phytochemical analysis of *Urginea indica* show the presence of minerals like Mg, Fe, Zn, flavonoids, alkaloids, antifungal glycoproteins, phenols, carbohydrates and saponins. In recent studies by using some generally accepted assays antioxidant properties in the bulb, roots, stems and leaves of *Urginea indica* are found. The naturally antioxidant chemicals like flavonoids, carotenoids, polyphenols are observed in *Urginea indica* (Pandey and Kumar, 2014). Antioxidant potential of extracted ethyl acetate, chloroform and methanol are reported in vitro. Whereas, extracted methanol from the bulb of the plant also show anti-cancerous properties. C-glycosyl flavonoid extracted from *Urginea indica* bulb

reported significant inhibition in the proliferation of Hepatic cancer cell lines (Babu *et. al.*, 2017). The C-glycosyl flavone has been reported as CDK-6 inhibitor. The in vitro study verified that CDK-6 is the chief target of flavonoid C-glycosyl. CDK-6 activity regulates the angiogenesis of the cancerous cells. The inhibition of CDK-6 activity linked to the apoptosis in cancer cells, which resulted in the reduction of cancerous cells (Babu *et. al.*, 2018).

The extracts of *Lantana camara* and *Urginea indica* have shown substantial phytochemical, antioxidant as well as anti-cancerous activities, so these herbal plants demand further scientific validation of their antioxidant and anticancer efficacy.

Plant Material

Lantana camera (leaves) and *Urginea indica* (whole plant) will be collected from Hamirpur, Himachal Pradesh, India and its identification will be confirmed by the Faculty of Botany, Abhilashi University, Mandi, Himachal Pradesh India.

Plant extract

The aqueous and ethanolic extracts of leaves of *Lantana camara* and bulb of *Urginea indica* will be prepared and subjected for experimental analysis.

Cell lines:

The following cell lines will be procured from National Centre for Cell Science (NCCS), Pune and used in this study.

- HepG2 (liver cancer) Einbonda *et. al.*, 2009
- MCF7 (breast cancer) Einbonda *et. al.*, 2009
- A549 (lung cancer) Tai et al., 2012

Thus, the aim and objectives are as follows:

C. Aims and objectives of the study:

Aims:

To evaluate the phytochemical, antioxidant and anti-cancerous activities of *Lantana camara* (LC) and *Urginea indica* (UI): ***in vitro*** approach.

Objectives:

- ❖ Determination of Phytochemical constituents and antioxidant activity.
- ❖ Determination of Phytochemical constituents of LC and UI extracts by chromatographic technique.
- ❖ Assessment of cytotoxic effect of LC and UI by *in vitro* study.
- ❖ Evaluate anti-cancerous activity of LC and UI extracts against cancerous cell lines.
- ❖ Molecular docking study of phytochemicals of LC and UI extract with possible target proteins.

D. Hypothesis

This investigation aims to provide experimental evidence for evaluation of antioxidant and anti-cancerous effects of *Lantana camara* and *Urginea indica extract* against cancer cell lines. This research work will also help to enhance the knowledge of traditional medicinal plants and provide scientific rationale for the traditional use of *Lantana camara* and *Urginea indica* in the management of cancerous diseases. Thus, the proposed work will help us to evaluate and confirm the efficacy of *Lantana camara* and *Urginea indica* and for the development of more appropriate anti-cancerous drugs this will deal as an advantage for the betterment of humankind.

E. Research Methodology:

Determination of Phytochemical constituents and antioxidant activity of plant extract

(a) Determination of Phytochemical constituents in aqueous and ethanolic extract of *Lantana camara* (LC) and *Urginea indica* (UI).

- **Qualitative analysis of phytochemicals:** Phytochemical analysis is the separation of different contents of the plants to identify their chemical constituents. These chemical compounds, like tannins, steroids, flavonoids, alkaloids and saponins etc., have medicinal importance. Chemical analysis will be carried out on the aqueous extract of the powdered specimens by using standard methods to identify their chemical contents, described by Edeoga (2005) and Faiza (2014).
- **Determination of total phenolic content by Folin-Ciocalteu phenolic**

reagent method: Phenolics compounds like simple phenols, phenolic acids, polyphenols, flavonoids, and tannins are the secondary metabolites of plants acting primarly as antioxidants. To determine the total concentration of phenolic hydroxyl groups in these plants extracts, an experimental confirmation procedure by using Folin and Ciocalteu's phenol agent i.e. Folin-c reagent will be performed. Polyphenols that are present in the plant extracts will react with specific Folin-Ciocalteu reagent, which can be quantified by spectral analysis (Blainski *et. al.,* 2013) .

- **Identification of Phytochemicals of extracts by analytical chromatographic technique (LC-MS):** Liquid chromatography-mass spectrometry (LC-MS) technique will be used for the determination of bioactive compounds present in the plants extracts (Vignesh *et al*., 2022).

(b) Assessment of antioxidant activity of LC and UI extracts

DPPH scavenging activity	Shimada *et al*., 1992)
ABTS scavenging activity	Re *et al*.,1999)
Hydrogen peroxide scavenging activity	Ruch *et al*., 1989)

(c) Assessment of cytotoxic activity of LC and UI by *in vitro study*

Determination of Cytotoxicity activity of LC aqueous extracts at three doses (50, 75, 100µg/mL).

Determination of Cytotoxicity activity of LC ethanolic extracts at three doses (50, 75, 100µg/mL).

Determination of Cytotoxicity activity of UI aqueous extracts at three doses (50, 75, 100µg/mL)

Determination of Cytotoxicity activity of UI ethanolic extracts at three doses (50, 75, 100µg/mL)

The most effective extract either Aqueous or Ethanolic of LC and UI assessed by *in vitro* study will be further used for anti-cancerous study in the experimental cell lines (*In vitro*).

(d) Assessment of anti-cancerous activity of plant extracts against cancerous cell

lines

Evaluate anti-cancerous properties of plant extract in cell lines by MTT assay.

Molecular docking study

Docking experiments will be carried out between the phytochemicals of the effective LC and UI extract and the target protein.

F. Data submission and Statistical Analysis

Values will be expressed in mean ± SD each group.

P value will be calculated using one-way ANOVA followed by Dunnett's test for multiple comparisons. Values of $p < 0.05$ will be considered significant in all cases.

ANNEXURE-1

***In vitro* study**

MTT assay [3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide] (Pareek *et al*., 2013)

Hepatic leakage markers (AST and LDH) in HepG2 cell line

ANNEXURE 2

LC-MS (Liquid chromatography-mass spectrometry)

Free radical scavenging activity of plant extract

- DPPH assay
- ABTS assay
- Hydrogen peroxide assay

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